UK National Reference Laboratory for Monitoring Bacteriological and Viral Contamination of Bivalve Molluscs Cefas, Weymouth

Annual Technical Summary Report for 2011/12

NRL for monitoring bacteriological and
viral contamination of bivalve molluscs
Cefas Weymouth Laboratory,
Barrack Road, The Nothe,
Weymouth, Dorset, DT4 8UB, UK
Telephone: +44 (0) 1305 206600
Fax: +44 (0) 1305 206601
E-mail: shellfishmicro.nrl@cefas.co.uk
Website: http://www.cefas.defra.gov.uk/nrl.aspx

SUMMARY TECHNICAL REPORT FOR THE UK NATIONAL REFERENCE LABORATORY FOR MONITORING BACTERIOLOGICAL AND VIRAL CONTAMINATION OF BIVALVE MOLLUSCS APRIL 2011 - MARCH 2012

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1. Introduction

The Centre for Environment, Fisheries and Aquaculture Science (Cefas) Weymouth is designated as the UK National Reference Laboratory (NRL) for monitoring bacteriological and viral contamination of bivalve molluscs. This report summarises the activities carried out by the NRL for the financial year 2011-12 according to the requirements of Regulation (EC) No. 882/2004 and as defined in the Service Level Agreement between the Food Standards Agency and Cefas. The description of activities included herein comprises co-ordination of UK Official Control Laboratories (OCL), provision of advice to the Competent Authority (CA) and collaboration with the European Union Reference Laboratory (EURL) through participation in comparative testing, research and development and representation at EURL workshops.

2. Co-ordination of the activities of the laboratories responsible for Official Control in the area of competence in the UK

2.1 Plan and organise proficiency tests between OCLs

i. Provision of proficiency tests

In October 2011 the NRL organised a distribution comprising Pacific oysters (*Crassostrea gigas*) and cockles (*Cerastoderma edule*) for enumeration of *Escherichia coli*. Whole matrix samples were provided to laboratories to test aspects of the methodologies not covered by the standard shellfish EQA scheme i.e. opening of shellfish and preparation of initial dilutions. Cockle matrix was included in the distribution for the first time. Material was distributed to 13 UK OCLs. Participation and performance assessments for *E. coli* for both samples are given in Tables 1, 2, and 3. Twelve (92%) OCLs returned results for this distribution; one laboratory did not examine the material as it was mistakenly left at the laboratory reception for an extended period. For *C. gigas* (RT42A) (Table 1) all laboratories that returned results reported *E. coli* MPN/100g within the expected range and received a maximum score of 12. Seven laboratories (53%) returned results for the cockle sample (RT42B) (Table 2) within ±3 theoretical standard deviations of the participant's median (expected range). One of these reported one replicate MPN value inconsistent with the tables given in ISO 7218 or those provided by the NRL. Five laboratories returned results either one or both replicates outside the participants median ±3 theoretical standard deviations, 1 laboratory returned a single replicate outside ±5 theoretical standard deviations of the median. The observed variability in laboratories results was discussed at the annual official control network meeting in November and a follow-up action plan agreed by the NRL. In brief follow-up actions included further statistical analyses of the dataset to examine the within and between laboratory variation, to include analysis of reference results obtained by the NRL. Examination of the time and temperature parameters to identify any possible impact of extended times or elevated temperature on participants' results. Evaluation the level of experience of laboratories within the network with respect to analysis of cockles relative to other bivalve species and additional practical activities amongst the network focusing on cockles. Follow-up activities will be reported separately.

Table 1. Summary of participation by OCLs – Sample RT 42A – Oysters

Participation summary - RT 42A	
Number of laboratories not returning results	1
Total participants reporting duplicate results for *E. coli* MPN	12
Participants reporting MPN results within the expected range[1]	12

[1]expected range = participants' median ± theoretical 3SD ($8.0 \times 10^1 - 4.9 \times 10^2$)

Table 2. Summary of participation by OCLs – Sample RT 42B – Cockles

Participation summary – RT 42B	
Total participants reporting duplicate results for *E. coli* MPN	12
Participants reporting MPN results within the expected range[1]	7
Participants reporting MPN results outside the expected range for one replicate	4
Participants reporting MPN results outside the expected range for both replicates	1
Participants reporting MPN result inconsistent to tube combination	1

[1]expected range = participants' median ± theoretical 3SD ($2.0 \times 10^1 - 5.4 \times 10^3$)

Table 3. Performance assessment of returned participants' results

Lab ID	RT 42A *E. coli* MPN/100g			RT 42B *E. coli* MPN/100g		
	Replicate 1	Replicate 2	Score	Replicate 1	Replicate 2	Score
7	230	170	12	310	330	10
9	80	50	12	40	130	9
14	230	80	12	80	230	12
48	330	110	12	1300	2400	9
67	110	170	12	20	50	9
97	330	490	12	230	330	12
145	-	-	-	-	-	-
166	230	80	12	460	1700	9
243	170	170	12	5400	3500	4
271	170	330	12	130	230	12
578	490	330	12	230	310	12
678	170	330	12	220	220	12
1160	140	130	12	270	790	12

ii. Provision of external quality assessment (EQA)

The performance of UK OCLs was subject to ongoing review according to the agreed scoring system. Formal performance assessments were undertaken for three distributions of the EURL/HPA EQA shellfish scheme (SF039, SF040 and SF041). Thirteen laboratories completed three distributions during this reporting period for *E. coli* enumeration and eleven for the detection of *Salmonella* spp. Laboratory performance is summarised in Tables 4 and 5. All laboratories that tested all three distributions achieved scores in excess of 70%, the measure of satisfactory performance.

Table 4. Performance of U.K. OCLs in Cefas/HPA EQA distributions for *E. coli*

Lab ID	SF039		SF040		SF041		Score	%
	SF0086	SF0087	SF0088	SF0089	SF0090	SF0091		
7	12	12	12	12	12	12	72	100
9	12	12	12	12	12	12	72	100
14	12	12	12	12	12	12	72	100
48	12	12	12	12	12	12	72	100
67	12	12	12	12	12	12	72	100
97	12	10	12	12	12	12	70	97
145	6	6	12	12	12	12	60	83
166	12	12	12	12	12	12	72	100
243	12	12	12	12	12	12	72	100
271	12	12	12	12	12	12	72	100
578	12	12	12	12	12	12	72	100
678	12	12	12	12	12	12	72	100
1160	7	12	12	12	12	12	67	93

Table 5. Performance of U.K. OCLs in Cefas/HPA EQA distributions for *Salmonella* spp.

Lab ID	SF039		SF040		SF041		Score	%
	SF0086	SF0087	SF0088	SF0089	SF0090	SF0091		
7	2	2	2	2	2	2	12	100
9	2	2	2	2	2	2	12	100
14	2	2	2	2	2	2	12	100
48	2	2	2	2	2	2	12	100
67	2	2	2	2	2	2	12	100
97	2	2	2	2	NE	2		
145	2	0	2	2	2	2	10	83
166	2	2	2	2	2	2	12	100
243	2	2	2	2	2	2	12	100
271	2	2	2	2	2	2	12	100
578	2	2	2	2	0	2	10	83
678	NE	NE	2	2	2	2		
1160	2	2	2	2	2	2	12	100

NE - not examined.

2.2 Supervision, liaison with and provision of advice to OCLs

i. Testing procedures

In April 2011, the NRL placed an updated generic protocol for the enumeration of *E. coli* in bivalve molluscs on the NRL website and informed the laboratory network of the update.

The NRL participated in the BSI Food Microbiology Committee and provided oral and written comment on standards relevant to the area of shellfish microbiology.

The NRL held separate liaison meetings with the Health Protection Agency and Public Health Wales to facilitate adoption of NRL protocols and advice and to ensure a consistent approach to sample transport and microbiological examination of shellfish samples.

The NRL produced and circulated a report entitled: "A comparison of homogenisation methods for bivalve molluscs prior to the enumeration of *Escherichia coli*.".

Ad hoc advice was given to individual laboratories on the following aspects of the microbiological examination of bivalve shellfish:

i. The application of minimum size requirements to bivalve shellfish received for microbiological examination.
ii. The effect of refrigeration of the first stage tubes in the enumeration of *E. coli* by the reference method.

The NRL undertook a pre-accreditation audit of shellfish testing at HPA London at the request of the laboratory.

ii. NRL website

The NRL website was migrated to the Defra domain as part of the rationalisation of Government websites. The new website address is: http://www.cefas.defra.gov.uk/nrl.aspx. Opportunity was taken to further review and update the website during and following this migration.

iii. NRL network meeting

The fourth network meeting of laboratories undertaking microbiological testing of bivalve shellfish was held at the NRL on the 16th and 17th November 2011. Twenty-two delegates attended the two day event. The first day (pm) comprised practical activities and seminars. Delegates were provided with hands on training in ISO 16649-2 (the TBX method) which had been recently approved for official control use for live bivalve molluscs by the European Union's Standing Committee on the Food Chain and Animal Health (SCFCAH). Seminars were presented by NRL staff and from the wider network on the affects of improved information technology on shellfish monitoring and surveillance, FSA funded studies on the prevalence, distribution and levels of norovirus titre in oyster harvesting areas in the UK and the use of remote sensing to predict vibrio risks. Topics covered on the second day included OCL performance in proficiency testing, reference and alternative methods for official control analyses, progress at ISO on the norovirus and hepatitis A virus standard and developments at the EU level including report of the 10th workshop of EU NRLs for monitoring bacteriological and viral contamination of bivalve molluscs.

3. Provision of assistance to the CA

3.1 Provision of technical advice and support to CA in development and management of the OCL network.

During 2011/12 a number of changes took place to the laboratory network and the NRL record of laboratories, together with their accreditation status and participation in proficiency testing, was updated. Information on changes was provided to the FSA. Liaison took place with the FSA on the inclusion, or otherwise, of two laboratories in the NRL laboratory network.

3.2 Dissemination to CA and OCLs of information provided by the EURL for monitoring bacteriological and viral contamination of bivalve molluscs.

Information disseminated by the EURL was provided to both the FSA and OCL via direct circulation of documentation. Specific papers circulated are listed below:

- Report on the 10th Workshop of NRLs for Monitoring Bacteriological and Viral Contamination of Bivalve Molluscs.
- Thematic Agenda of 11th Workshop of Microbiological NRLs, 24th - 26th April 2012.
- Report of an EURL proficiency test distribution for *E. coli* in bivalve molluscs
- Report of an EURL proficiency test distribution for norovirus and hepatitis A

3.3 Other advice

The NRL gave advice to FSANI on conditions under which shellfish could support the growth of micro-organisms.

The NRL produced and distributed a report entitled “Real-time RT-PCR results for norovirus in oysters; the relationship between Ct values and copies/g digestive tissues.”.

4. Collaboration with the EURL for monitoring bacteriological and viral contamination of bivalve molluscs - Participation in proficiency testing (PT) and method validation studies organised by the EURL.

4.1 Participation in EURL PT for norovirus and hepatitis A.

The NRL participated in two proficiency testing distributions organised by the EURL for norovirus and hepatitis A virus in 2011 (March/April [RT39] and December [RT43]). Both distributions comprised matrix and laboratory constructed samples (4 and 7 samples). The NRL scored 100% for all performance measures (relative accuracy, specificity and sensitivity).

4.2 Participation in EURL/ HPA EQA shellfish scheme for E. coli *and* Salmonella *spp.*

Performance assessments for EURL/HPA EQA shellfish scheme for *E. coli* and *Salmonella* spp. were undertaken for distributions SF039, SF040 and SF041 in 2011/12. The results obtained by the UK NRL were assessed together with all other participants. The UK NRL achieved a rolling performance assessment of >70% for *E. coli* enumeration and *Salmonella* spp. detection.

4.3 Participation in EURL supplementary PT for E. coli *and* Salmonella *spp.*

The NRL participated in the EURL PT distribution for *E. coli* enumeration and detection of *Salmonella* spp. in whole Pacific oysters (*Crassostrea gigas*) in November 2011. The NRL achieved performance assessment of 100%.

4.4 Participation in EURL interlaboratory Vibrio *spp. study.*

The NRL participated in an interlaboratory trial for the generation of additional data for the revision of ISO TS 21872 “Microbiology of food and animal feeding stuffs – Horizontal method for the detection of potentially enteropathogenic *Vibrio* spp. Part 1 Detection of *Vibrio parahaemolyticus* and *Vibrio cholerae*, and Part 2 Detection of species other than *Vibrio parahaemolyticus* and *Vibrio*

cholera organized by the EURL. The study provided comparative data on a selection of PCR based species and pathogenicity identification targets and conventional biochemical species characterization tests. The NRL performed well, correctly identifying all distributed reference strains to the species level and accurately assigning pathogenicity markers of *V. parahaemolyticus* although some differences in the performance of primer sets prescribed by the EURL were observed. Specifically, discordant results were observed between primer sets for sample designated EURLV05/14: it was noted that primers designated VP21/22 (Lee and Pan 1993) targeting the *tdh* gene of *V. parahaemolyticus* did not enable detection of TDH in the sample whereas those recommended by Bej *et al* (1999) indicated the presence of this toxigenic marker gene. Primer sets designated S1/S2 (Suthienkul *et al* 1995) did not enable detection of *trh* gene in the same sample whereas the *trh*1,2 primers of Bej *et al* (1999) identified the presence of *trh*. EURL strain EURLV05/14 was subsequently confirmed in the report of RT38 as *tdh* and *trh* positive.

Bej,A.K.; Patterson,D.P.; Brasher,C.W.; Vickery,M.C.L.; Jones,D.D.; Kaysner,C.A., 1999. Detection of total and hemolysin-producing *Vibrio parahaemolyticus* in shellfish using multiplex PCR amplification of *tl*, *tdh* and *trh*. *Journal of Microbiological Methods* 36, 215 – 225.

Lee,C.Y.; Pan,S.F., 1993. Rapid and specific detection of the TDH gene in *Vibrio parahaemolyticus* by the polymerase chain reaction. *Journal of General Microbiology* 139, 3225 – 3231.

Suthienkul,O.; Ishibashi,M.; Iida,T.; Nettip,N.; Supavej,S.; Eampokalap,B.; Makino,M.; Honda,T., 1995. Urease production correlates with possession of the *trh* gene in *Vibrio parahaemolyticus* strains isolated in Thailand. *Journal of Infectious Diseases* 172, 1405 – 1408.

4.5 Participation in HPA EQA for pathogenic Vibrio *spp.*

The NRL participated in the HPA FEPTU *Vibrio* scheme. Four samples were analysed across two distributions in November 2011 and March 2012 (V033 and V034). The NRL results for detection of *V. parahaemolyticus* and *V. cholerae* were satisfactory on both occasions for both samples.

4.6 Participation in EURL pre-validation studies in support of the development of new analytical methods for the detection of viruses in shellfish.

The NRL maintained accreditation for the CEN method. The ongoing competency of analysts to carry out the method has been assessed. In-house comparisons were conducted of the performance of the CEN norovirus method for oysters and mussels. Initial investigations into the linearity of the CEN norovirus method were conducted.

4.7 Meetings, workshops and task forces

The NRL director and a virology specialist participated in the 10th annual workshop of NRLs for monitoring bacteriological and viral contamination of bivalve molluscs held in Weymouth in May 2011. A report detailing participation and major outcomes was provided to the FSA and the laboratory network following the workshop. No other meetings were organised by the EURL.

R. J. Lee

Dr Ron Lee
NRL Director

Date…30/04/12……

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Head office
Centre for Environment,
Fisheries & Aquaculture Science
Pakefield Road, Lowestoft,
Suffolk NR33 0HT UK

Tel +44 (0) 1502 56 2244
Fax +44 (0) 1502 51 3865
Web www.cefas.defra.gov.uk

Centre for Environment,
Fisheries & Aquaculture Science
Weymouth Laboratory,
Barrack Road, The Nothe, Weymouth,
Dorset DT4 8UB

Tel +44 (0) 1305 206600
Fax +44 (0) 1305 206601

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